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Muscle sympathetic activity in resting and exercising humans
with and without heart failure

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1 ABSTRACT

2 The sympathetic nervous system is critical for coordinating the cardiovascular response
3 to various types of physical exercise. In a number of disease states, including human heart failure
4 with reduced ejection fraction (HFrEF), this regulation can be disturbed and adversely affect
5 outcome. The purpose of this review is to describe sympathetic activity at rest and during
6 exercise in both healthy humans and those with HFrEF and outline factors which influence these
7 responses. We focus predominately on studies which report direct measurements of efferent
8 sympathetic nerve traffic to skeletal muscle (muscle sympathetic nerve activity; MSNA) using
9 intraneural microneurographic recordings. Differences in MSNA discharge between subjects
10 with and without HFrEF both at rest and during exercise and the influence of exercise training on
11 the sympathetic response to exercise will be discussed. In contrast to healthy controls, in HFrEF
12 MSNA increases during mild to moderate dynamic exercise. This increase may contribute to the
13 exercise intolerance characteristic of HFrEF by limiting muscle blood flow and may be
14 attenuated by exercise training. Future investigations are needed to clarify the neural afferent
15 mechanisms which contribute to efferent sympathetic activation at rest and during exercise in
16 HFrEF.

17 Keywords: exercise, heart failure, HFrEF, microneurography, muscle sympathetic nerve activity,
18 exercise training, peak oxygen uptake

1 INTRODUCTION

2 The sympathetic nervous system plays an essential role in regulating and coordinating the
3 reflex cardiovascular response to exercise in both health and disease (Mitchell, 1990). A variety
4 of methods are used to measure sympathetic nervous system activation in humans. These range
5 from non-invasive techniques such as spectral analysis of heart rate variability (Task Force of the
6 European Society of Cardiology and North American Society of Pacing and Electrophysiology,
7 1996; Notarius et al., 1999b) to quantitation of plasma norepinephrine (NE) (Jensen et al., 1994),
8 total NE spillover (Esler et al., 1988) or spillover across specific organs such as the heart, kidney
9 and skeletal muscle (Kingwell et al., 1995; Dinunno et al., 1999; Grassi and Esler, 1999). By
10 contrast, with microneurography, efferent sympathetic nervous discharge can be recorded
11 directly from the innervation of blood vessels in skeletal muscle or skin (Valbo et al., 1979).

12 Muscle sympathetic nerve activity (MSNA) is pulse synchronous and expressed as either
13 number of bursts per minute (frequency) or multi-unit number of bursts per 100 heart beats
14 (incidence) or total MSNA which integrates burst amplitude (normalized burst area) (Valbo et
15 al., 2004). Measurements of MSNA offer a more sensitive method of detecting and quantifying
16 sudden changes in sympathetic nervous outflow than do plasma NE concentrations (Floras et al.,
17 1986; Grassi et al., 2008). Further, unlike the determination of NE spillover, changes in MSNA
18 appear immediately rather than after a lag time of minutes. Simultaneously acquired sympathetic
19 nerve recordings to different muscle groups are congruent both at rest and when elevated in
20 response to the application of lower body negative pressure, implying that a recording from one
21 location represents sympathetic outflow to muscle in general (Rea and Wallin, 1989). Because
22 MSNA represents the efferent limb of the skeletal muscle reflex arc, it is of particular interest to

1 those investigating responses to physical exercise. Importantly, to do so requires a stationary
2 limb.

3 Sympathoexcitation, or elevated sympathetic activity at rest, is a hallmark of untreated
4 patients with heart failure due to reduced ejection fraction (HFrEF) (Leimbach et al., 1986).
5 When present, it is associated with an adverse prognosis (Barretto et al., 2009). Abnormal
6 sympathetic control may also contribute to the exercise intolerance observed in HFrEF. Our
7 group was the first to report a relationship between the extent to which MSNA is increased in
8 HFrEF patients and their level of exercise intolerance, independent of left ventricular ejection
9 fraction (LVEF) (Notarius et al., 1999a; Notarius et al., 2014).

10 In this review we will focus on the response of efferent muscle sympathetic nerve traffic to
11 static and dynamic exercise in healthy subjects and patients with HFrEF. To frame this
12 discussion we first will summarize known differences in resting MSNA between these two
13 groups. Second, we will discuss sympathetic overactivity at rest in HFrEF and how it impacts
14 exercise tolerance. Third, we will review the efferent sympathetic response to acute static and
15 dynamic exercise in HFrEF and identify possible key mechanisms. In both healthy control and
16 HFrEF cohorts, we will briefly evaluate the effect of exercise training on sympathetic activity.
17 Finally, we will identify potential future research directions.

18

19 SYMPATHETIC ACTIVITY IN HEALTHY SUBJECTS

20 *REST*

Age and sex are two major influences on resting sympathetic nerve activity in healthy subjects (Ng *et al.*, 1993). Muscle sympathetic firing rates are generally low in young healthy adults (Floras and Hara, 1993). Across all ages, MSNA is higher in men than women (Seals and Esler, 2000). Interestingly, these age and sex differences in MSNA are not reflected in concurrent measurements of plasma NE concentrations (Seals and Esler, 2000), underscoring the limitation of this indirect assay for between- subject comparisons of sympathetic activity.

Sympathetic outflow to arterial resistance vessels is greater in healthy older compared with younger men. This impacts vasoconstrictor tone; older men have higher vascular resistance and lower conductance, which is its inverse (Hart *et al.*, 2009). There is a significant negative correlation between femoral blood flow or vascular conductance (flow/pressure) and MSNA burst frequency in men (Dinenno *et al.*, 1999) but not women (Moreau *et al.*, 2003). These observations belie the common assumption that reductions in vascular conductance always reflect sympathoexcitation (Seals and Esler, 2000; Hart *et al.*, 2009). We reported a similar disassociation between vascular resistance (pressure/flow) in the forearm and MSNA in middle-aged fit men, but a positive relationship between the two variables in similarly aged sedentary men (Notarius *et al.*, 2012). In those experiments MSNA was increased reflexively by applying increasing levels of lower body negative pressure to reduce central venous pressure and unload gradually the arterial baroreceptors.

In addition to age, sex and fitness, posture and body weight also can affect resting MSNA. MSNA is lower in young healthy subjects in the supine vs upright posture (Ray *et al.*, 1993). This difference was explained by higher central venous pressure, implicating stimulation of cardiopulmonary baroreceptors as the responsible reflex sympathoinhibitory mechanism. Obesity also is associated with a marked sympathetic activation: MSNA of young normotensive

obese individuals is approximately double that of their non-obese control counterparts even in the absence of coexisting sleep apnea (Grassi et al., 2014).

Repeat microneurographic recordings in a single healthy individual over time yield generally reproducible values (Valbo et al., 2004). However, even when the influence of these identified factors is accounted for, MSNA at rest can vary considerably between subjects, particularly in women (Hart et al., 2009). At the vascular level, an increased neurogenic stimulus to vasoconstriction may be offset by diminished alpha adrenoceptor responsiveness. For example, Charkoudian et al. (2006) reported that healthy young men and women with higher MSNA at rest had less vasoconstriction in response to both exogenous NE as well as NE release stimulated by intrabrachial tyramine, and concluded that such inter-individual variability in vascular adrenergic responsiveness could offset increased central sympathetic drive so as to maintain normal blood pressure regulation.

Whether this healthy equilibrium between the impact of alterations in resting MSNA and adrenergic responsiveness on vascular resistance is altered with age, posture, body mass, or in conditions such as hypertension and heart failure is an important subject for further research.

EXERCISE

In healthy subjects, sympathetic activity increases during dynamic exercise in order to redistribute blood flow to the working muscles and increase oxygen delivery (Mitchell, 1990; Hasking et al., 1988). MSNA responses during exercise were first studied using a handgrip model. Sympathetic nerve activity was recorded from the peroneal (fibular) nerve of a stationary leg during either static or dynamic exercise of various intensities.

The muscle metaboreflex is the primary sympathoexcitatory mechanism elicited by static exercise (Mark et al., 1985; Hansen et al., 1994). Its potency is revealed by the post-handgrip ischemia manoeuvre. This traps within the forearm the metabolites released by muscle contraction, prolonging stimulation of type III and IV muscle afferents. This manoeuvre was designed to eliminate any confounding influence of both central volitional effects and the mechanical effect of muscle contraction (muscle mechanoreflex) on MSNA (Mark et al., 1985). MSNA is increased during the second minute of static handgrip at an intensity of 30% or greater of maximum voluntary contraction (MVC) whereas dynamic handgrip shows no such elevation in MSNA unless flow is diminished by vascular occlusion during exercise (Victor et al., 1987). This is considered analogous to the low flow state evident in HFrEF patients. Thus, sympathetic responses are intensity- and exercise mode-dependent, with the muscle metaboreflex active during both dynamic and static handgrip, (Hansen et al., 1994; Mark et al., 1985) and maintained during post-handgrip ischemia (Victor et al., 1987). However, data by others who observed an early increase in MSNA during dynamic handgrip and a prompt return to baseline during post-exercise ischemia, emphasize an important contribution of the muscle mechanoreflex to the net MSNA response (Batman et al., 1994).

A more recent study by Stickland (2008), involving healthy young subjects, demonstrated that the increase in MSNA in response to dynamic handgrip exercise at 50% MVC could be reduced when the subject was breathing 100% oxygen, whereas there was no effect at rest, suggesting that the arterial chemoreflex contributes importantly to the sympathoexcitatory response to dynamic handgrip (Stickland and Miller, 2008). In a subsequent study using a different exercise mode, changes in blood flow and conductance with leg exercise during

1 hyperoxia followed a similar time course as the previous reductions in MSNA (Stickland et al.,
2 2011).

3 A leg extension model, engaging a much larger muscle mass than the forearm, was
4 developed to study blood flow responses during two or one-legged exercise. This made it
5 possible to measure MSNA in the contralateral leg during single leg extension under both
6 dynamic (isotonic) or static (isometric) conditions (Andersen et al., 1985). Using this model, Ray
7 and colleagues found a decrease in MSNA with upright mild intensity exercise (20, 30 and 40
8 watts), rather than the increase anticipated from previous static and dynamic handgrip data. This
9 decrease was greatest at the lowest work rate (Ray et al., 1993). Ray et al. also measured MSNA
10 during a 30 watt work rate while recording central venous pressure (CVP) and compared supine
11 to upright posture. In the upright posture, resting CVP was lower compared with supine yet
12 increased during exercise due to central translocation of blood from the mechanical pumping
13 effect of rhythmic muscle contractions with exercise. There was a drop in CVP and no change in
14 MSNA with subjects supine, but an increase in CVP and a fall in MSNA when they were
15 upright. The latter was attributed to stimulation of sympathoinhibitory cardiopulmonary
16 baroreceptors by the former (Ray et al., 1993).

17 Concurrently, Saito and Mano (1991) demonstrated that the mode of leg exercise affects
18 the MSNA response. They compared one-legged cycling at up to 30% of peak oxygen uptake
19 ($\text{VO}_{2\text{peak}}$) to one-legged static leg extension and measured MSNA in the tibial nerve of the
20 contralateral resting leg. They showed a work rate- related decrease in MSNA during mild
21 exercise up to 25 watts but not at 50 watts, whereas MSNA significantly increased during static
22 leg exercise performed at 20% MVC (Saito and Mano, 1991). Subsequent experiments involving
23 MSNA recorded from the median nerve of the arm during bilateral cycling showed a drop in

1 burst frequency at work rates less than 40% of $\text{VO}_{2\text{peak}}$ but an increase at rates of 60 and 74%
2 $\text{VO}_{2\text{peak}}$ (Saito et al., 1993).

3 Using the same model, this group (Katayama et al., 2011) and others (Ichinose et al.,
4 2008) confirmed that sympathetic nerve traffic in the median nerve of the arm is diminished at
5 both absolute and relative mild to moderate work rates, but increased during heavy or exhaustive
6 leg cycling (Ichinose et al., 2008). An increase in MSNA at 40% of $\text{VO}_{2\text{peak}}$ occurred only
7 when subjects were breathing a hypoxic gas mixture of 12.7% oxygen. These findings may have
8 implications for those HFrEF patients with compromised oxygen delivery during exercise
9 (Esposito et al., 2010). The literature regarding MSNA responses to exercise in healthy subjects
10 is summarized in Table 1.

11 In sum, MSNA during exercise in healthy subjects varies according to type and intensity
12 of exercise as well as the amount of active muscle mass. In contrast to static exercise, mild to
13 moderate dynamic exercise yields a drop in MSNA with increases above baseline only at
14 intensities exceeding 40 % of $\text{VO}_{2\text{peak}}$. MSNA responses under specific exercise conditions are
15 based on the net response of the individual afferent reflexes recruited and the involvement of
16 feed-forward central command.

17 *EXERCISE TRAINING*

18 In healthy sedentary subjects, six weeks of high intensity endurance exercise training had
19 no effect on resting MSNA, but the anticipated drop in MSNA burst frequency during moderate
20 intensity one-legged cycling became markedly attenuated by the third minute of exercise (Ray,
21 1999). The absence of any change in resting MSNA after endurance training is a consistent
22 finding in the literature (Carter and Ray, 2015) and is compatible with cross-sectional data

reporting no difference in resting MSNA between trained and untrained young (Seals, 1991; Svedenhag et al., 1984), middle-aged, or older healthy subjects (Sheldahl et al., 1994; Notarius et al., 2012). In contrast, elite endurance athletes exhibited an increase in resting MSNA compared to sedentary individuals who were matched for adiposity (Alvarez et al., 2005). This observation highlights the importance of considering the potential confounding influence of body mass when comparing trained and untrained subjects. Others have also showed a decrease in MSNA during both isometric (Somers et al., 1992) and non-fatiguing dynamic handgrip (Sinoway et al., 1996) following unilateral forearm endurance training.

In contrast, the response to resistance training is less consistent. Saito and colleagues found that MSNA actually increased following 4 weeks of maximal handgrip training, although it reverted to baseline after 4 weeks of detraining (Saito et al., 2009). This result is consistent with cross-sectional data showing higher MSNA at rest in bodybuilders compared with sedentary controls (Sinoway et al. 1992). However, others have reported no change at rest either following isometric handgrip training (Ray and Carrasco, 2000), or whole body resistance training (Carter et al., 2003). Also, 4 weeks of submaximal intensity static handgrip training had no effect on the magnitude of increase in MSNA during handgrip (Saito et al., 2009).

Thus endurance exercise training in healthy subjects lowers MSNA both at rest and during exercise, but from the limited and discordant data available it is less certain whether resistance exercise training achieves similar effects.

SYMPATHETIC ACTIVITY IN HEART FAILURE

REST

1 In many treated heart failure patients with relatively preserved exercise capacity, MSNA
2 when measured at rest is similar to that of age and sex matched healthy controls (Notarius et al.,
3 2001). However, the majority of patients with HFrEF have elevated resting sympathetic activity.
4 This, is now recognized to be an independent predictor of premature mortality (Barretto et al.,
5 2009; Cohn et al., 1984; Kaye et al., 1995; Petersson et al., 2005). The magnitude and time
6 course of elevated sympathetic outflow across all vascular beds is not uniform but organ specific
7 (Rundqvist et al., 1997). An initial elevation in cardiac NE spillover precedes a later doubling of
8 skeletal muscle, total body and renal NE spillover (Rundqvist et al., 1997). The relative increase
9 in cardiac NE spillover exceeds that of renal NE spillover (Hasking et al., 1986). Importantly, the
10 magnitude of such sympathoexcitation is independent of the degree of LVEF impairment
11 (Ferguson et al., 1990; Notarius et al., 1999a; Notarius et al., 2001).

12 Most HFrEF patients have exercise intolerance, as evidenced by reduced predicted peak
13 oxygen consumption (VO_{2peak}) relative to healthy controls (Pina et al., 2003). Lower VO_{2peak}
14 also has been identified as an important marker of poor outcome (Stelken et al., 1996)
15 independent of the extent of left ventricular dysfunction (Franciosa et al., 1981; Cohn et al.,
16 1993). Figure 1 depicts representative MSNA recordings from 2 healthy subjects, one older
17 (upper left panel) and 2 HFrEF patients of similar age but with different exercise capacities. This
18 Figure demonstrates the variability in resting MSNA observed normally in healthy subjects
19 (most likely age-related), as well as the relationship between exercise capacity and resting
20 MSNA in HFrEF subjects. MSNA burst frequency is elevated markedly in the HFrEF patient
21 with poor exercise tolerance, compared with the relatively normal values present in the HFrEF
22 patient with preserved exercise capacity (Figure 1, right panels).

Our group was the first to report a link between $\text{VO}_{2\text{peak}}$ and resting MSNA in HFrEF (in contrast to age-matched healthy control subjects), raising the hypothesis of a peripheral neurogenic limit to exercise tolerance in this patient population (Notarius et al., 1999a). We subsequently demonstrated that the inverse relationship in HFrEF patients was specific to skeletal muscle in that $\text{VO}_{2\text{peak}}$ and cardiac NE spillover showed no such association (Notarius et al., 2002). Patients with an ischemic cardiomyopathy (ICM) etiology have a significantly higher mean MSNA burst frequency compared with those of non-ischemic or dilated etiology; although both are higher than mean values for age-matched healthy subjects (Notarius et al., 2007). This could reflect, consistent with our previous work (Notarius et al., 2001), the generally lower values for peak VO_2 of ICM patients (Notarius et al., 2007), or represent a reflex sympathoexcitatory response to chronic myocardial ischemia (Floras 2009).

To explain the elevated sympathetic activity present in most HFrEF patients, we and others have proposed several reflex mechanisms involving either augmentation of existing sympathoexcitatory and/or reduced sympathoinhibitory influences, (Azevedo et al., 2000; Diberner Dunlap et al., 1996; Floras, 2001; Floras and Ponikowski, 2015; Millar et al., 2015; Notarius et al., 2001; Schultz et al., 2007). The assumption of a heart failure-induced impairment in arterial baroreflex inhibition was disproved by our discovery of muscle sympathetic alternans which demonstrated that the arterial baroreceptor control of MSNA was preserved in HFrEF patients (Ando et al., 1997b; Floras, 2001). By contrast there is strong evidence for impaired cardiopulmonary baroreflex inhibitory modulation of MSNA (Diberner Dunlap et al., 1996). However, in some individuals paradoxical vasodilation can occur when the cardiopulmonary baroreceptors are unloaded experimentally by lower body negative pressure (Notarius et al., 2009). Our group has previously identified a cardiac-specific sympathoexcitatory reflex related

to increased filling pressure in HFrEF (Azevedo et al., 2000) and recently published evidence supporting an augmented excitatory cardiopulmonary-MSNA reflex response to increased preload which incorporates two distinct single-unit populations with differing firing properties (Millar et al., 2013; Millar et al., 2015). There is also increasing evidence to support overactivity of the muscle metaboreflex (Notarius and Floras, 2007; Notarius et al., 2001; Piepoli and Coats, 2007) and arterial chemoreflex (Despas et al., 2012) at rest in patients with HFrEF compared with age-matched controls. Coexisting sleep apnea in HFrEF patients also augments their daytime MSNA, independent of HFrEF etiology (Spaak et al., 2005), but can be inhibited when such patients are treated with nocturnal continuous positive airway pressure (Usui et al., 2005). This supports the concept, recently reviewed by Abboud and Kumar (2014), that persistent daytime elevation of MSNA can result from neuroplastic changes in chronic chemoreceptor sensitivity and in central neural modulation of sympathetic outflow, and that such changes are reversible if obstructive sleep apnea is abolished.

In summary, elevated MSNA at rest in HFrEF patients is related to the extent of exercise intolerance and is independent of the severity of left ventricular dysfunction. Each of ischemic etiology, elevated cardiac preload, and the presence of sleep apnea can contribute to increases in resting MSNA in this population. This elevated sympathetic activity at rest reflects the integration of central neural changes, impaired sympathoinhibitory reflex afferents in response to reduced stroke volume, and excessive sympathoexcitatory compensatory reflex output to the brainstem. The relative importance of each factor can vary from patient to patient and remains an active area of investigation.

EXERCISE

1 The most commonly applied exercise paradigm to study MSNA in HFrEF has been
2 handgrip. MSNA is elevated during static handgrip (usually performed at 30% MVC) compared
3 with age-matched controls (Sterns et al., 1991; Murai et al., 2009). This increase has been
4 attributed in part to the increased probability of multiple firing of single sympathetic fibers
5 (Murai et al., 2009).

6 The functional consequences of augmented sympathetic responses during exercise
7 include exaggerated peripheral neurogenic vasoconstriction (Crisafulli et al., 2007) and impaired
8 vasodilation (Alves et al., 2007). We hypothesize that in such after-load sensitive patients,
9 exaggerated vasoconstriction in response to muscle metaboreflex activation during dynamic
10 handgrip exercise supports blood pressure, but limits muscle perfusion and thus exercise
11 tolerance. This contrasts with healthy subjects who rely more on increases in cardiac output to
12 elevate blood pressure during exercise. In a dog model of heart failure, metaboreflex activation
13 resulted in a vasoconstriction-mediated rather than a flow-mediated increase in blood pressure
14 (Hammond et al., 2000). Alves et al. (2007) reported that, in contrast to healthy control subjects,
15 patients with advanced heart failure exhibited a blunted reflex vasodilatory response to static
16 handgrip. This was reversed by an intra-arterial infusion of the alpha blocker, phentolamine.
17 Similarly, phentolamine infusion increased muscle vasodilation during mental stress in HFrEF
18 patients (Santos et al., 2005) and reversed paradoxical vasoconstriction observed during hypoxia
19 (Alves et al., 2012). Thus, sympathoexcitation can functionally exaggerate vasoconstriction and
20 impair vasodilation in HFrEF.

21 We have recorded MSNA during two minutes of both static and mild, moderate and
22 intense levels of dynamic handgrip in HFrEF and age-matched control subjects. To isolate the
23 muscle metaboreflex handgrip was followed immediately by post-exercise circulatory arrest.

1 MSNA increased in response to handgrip exercise in HFrEF compared with age-matched healthy
2 subjects. The magnitude of this increase differed with exercise mode, intensity, and the patients'
3 baseline fitness level (Notarius et al., 2001). HFrEF patients had an increase in MSNA at a lower
4 threshold than healthy controls during dynamic and static handgrip. This difference was
5 maintained during post-handgrip ischemia but following only static handgrip and the highest
6 intensity of dynamic handgrip (50% MVC). These findings support a role for the muscle
7 metaboreflex in eliciting sympathoexcitation at these levels of exercise (Notarius et al., 2001)
8 and are consistent with previous observations, made both during and following exhaustive
9 dynamic handgrip at 25% MVC (Silber et al., 1998). Others have reported that MSNA returns to
10 baseline during post-handgrip ischemia after static handgrip (Sterns et al., 1991) and lower
11 intensity dynamic handgrip (20% MVC), but is increased during passive exercise, and concluded
12 that it is rather muscle mechanoreflex activation that augments MSNA in HFrEF during low
13 intensity exercise (Middlekauff et al., 2004; Middlekauff and Sinoway, 2007). Importantly, our
14 protocol did not elicit an immediate exercise-induced increase in MSNA. MSNA rose only
15 during the second minute of handgrip, consistent with a primary role for the metaboreflex,
16 particularly during dynamic handgrip in HFrEF (Notarius et al., 2001; Notarius and Floras,
17 2007). Differences between studies with respect to subjects' posture during exercise or their co-
18 morbidities, especially diabetes, could explain these discordant results.

19 Although it is a pragmatic model, handgrip is not representative of the bulk of real-life
20 day to day physical activity which usually engages much larger muscle mass. However, at
21 present there are limited data available concerning the effects of leg exercise on sympathetic
22 activity in HFrEF. Total body NE spillover is elevated in HFrEF compared to control subjects
23 during both bilateral cycling and one-legged dynamic leg extension exercise modes (Esposito et

al., 2010). Infusion of clonidine, a centrally and peripherally acting alpha-2 receptor agonist, lowers cardiac NE and plasma NE concentrations and increases leg blood flow during dynamic exercise in HFrEF (Azevedo et al., 1999; Lang et al., 1997). However in a comparative study, patients with HFrEF experienced less sympathoinhibition with clonidine compared to healthy controls suggesting decreased central alpha-2 adrenergic sensitivity in HFrEF (Lang et al., 1997). This may result in less prejunctional alpha-2 mediated inhibition of NE release both centrally and at the peripheral sympathetic nerve terminal, providing an additional mechanism for greater neurogenic vasoconstriction in HFrEF. There also may be up-regulation of arterial alpha-1 adrenoceptors with increased vasoconstrictor sensitivity, as has been demonstrated in a paced canine model of HFrEF (Forster et al., 1989; Forster and Armstrong, 1990). However in human heart failure the transduction of neural input into vascular responses, quantified as the gain of the cross-spectral transfer function between oscillations in MSNA and oscillations in blood pressure, is attenuated relative to healthy matched control subjects (Ando et al. 1997a).

These altered patterns of sympathetic response in HFrEF during submaximal dynamic handgrip exercise and peak leg exercise led us to measure MSNA in one-leg in HFrEF patients while the contralateral leg performed mild and moderate intensity dynamic cycling exercise. Similar to what we had observed previously at rest, we found an inverse relationship between MSNA burst frequency during moderate submaximal leg cycling exercise and peak VO₂ in subjects with and without HFrEF, independent of resting MSNA (Notarius et al., 2014) (Figure 2). This link between MSNA during dynamic exercise and peak exercise capacity gives further support to the concept of a neurogenic limit to exercise in HFrEF. This relationship was not observed by others during static handgrip exercise (Kuniyoshi et al., 2014). Further, we hypothesized that, in contrast to the drop in MSNA observed previously in young healthy control

1 subjects during dynamic one-legged cycling (Ray et al., 1993), MSNA would increase in patients
2 with HFrEF. Indeed, we found divergent MSNA responses to both 2 minutes of mild (unloaded)
3 and moderate (50% of $\text{VO}_{2\text{peak}}$) one-legged cycling, in HFrEF compared with age-matched
4 control subjects. Despite having similar heart rate responses MSNA burst frequency increased
5 during both intensities of exercise in HFrEF patients but fell in control subjects, (Notarius et al.,
6 2015) (Figure 3). These recent results provided the first direct evidence of increased sympathetic
7 outflow to the calf muscle during dynamic leg exercise in HFrEF, and were linked to reduced
8 exercise capacity.

9 The current literature regarding MSNA responses to exercise in HFrEF cohorts is
10 summarized in Table 2. In sum, MSNA during handgrip exercise in HFrEF is activated by a
11 lower intensity threshold compared with age-matched control subjects, whereas the exercise-
12 induced response to mild and moderate dynamic leg cycling is qualitatively different from
13 healthy controls. The functional consequence of such sympathoexcitation is likely peripheral
14 vasoconstriction which could directly impact exercise tolerance by limiting skeletal muscle
15 blood flow in HFrEF. The precise mechanism for the divergent MSNA responses to dynamic leg
16 exercise requires further investigation but is likely to involve recruitment of sympathoexcitatory
17 reflexes (muscle metabo- and mechano-, cardiopulmonary baro- and arterial chemoreceptor
18 reflexes).

19
20 *EXERCISE TRAINING*

21 Neural adaptations following a single bout of exercise might anticipate the response to
22 exercise training (Floras et al., 1989). Almost twenty years ago, our group compared MSNA
23 after moderate intensity treadmill exercise in young HFrEF patients with dilated cardiomyopathy

1 to that of age-matched controls. Despite marked peripheral vasodilation post-exercise, we found
2 no reflex increase in MSNA in either group, suggesting acute arterial baroreceptor reflex
3 resetting. Further, stroke volume and systolic blood pressure were higher post-exercise in HFrEF
4 patients (consistent with the failing ventricle's response to afterload reduction) but not in
5 controls. These findings suggested that by lowering peripheral resistance and increasing stroke
6 volume, exercise training might be an important non-pharmacological therapy for HFrEF
7 patients (Hara and Floras, 1996).

8 The specific effect of exercise training on resting and exercise MSNA in HFrEF patients
9 has received limited attention. In a series of studies, 4 months of exercise training (25-40 minutes
10 of cycling exercise plus warm up and cool down, 3 times per week at a heart rate of 10% less
11 than anaerobic threshold) in patients with HFrEF normalized resting MSNA and reduced forearm
12 vascular resistance (Roveda et al., 2003; Fraga et al., 2007). This response was independent of
13 both age (Antunes-Correa et al., 2012) and gender (Antunes-Correa et al., 2010). However, only
14 one study by Soares-Miranda et al., also from this group, recorded MSNA during exercise
15 (Soares-Miranda et al., 2011). They compared MSNA and forearm vascular resistance responses
16 during static handgrip in age-matched controls and two HFrEF groups, one untrained and the
17 other before and after 4 months of dynamic exercise training. Elevated MSNA during static
18 handgrip in the untrained HFrEF group was normalized by exercise training. The authors
19 attributed this drop to the reduction in baseline MSNA. Such a reduction has important
20 implications for improving outcomes in this patient population. However, whether this training
21 response occurs during dynamic leg exercise in HFrEF, which is a more clinically relevant
22 intervention, is unknown. This remains a fertile area for investigation.

1 **FUTURE DIRECTIONS**

2 Evidence of elevated MSNA both at rest and during dynamic leg exercise in HFrEF, in
3 contrast to healthy control subjects, now sets the stage for future studies to elucidate the
4 mechanisms responsible for such sympathoexcitation. Diminished sympathoinhibitory
5 mechanisms such as the cardiopulmonary baroreflex and enhanced excitatory reflexes such as
6 the muscle metaboreflex, mechanoreflex, and arterial chemoreceptor must be systematically
7 evaluated with respect to their role in the augmented sympathetic response to dynamic leg
8 exercise in HFrEF patients. Whether patients with heart failure with preserved ejection fraction
9 (HFpEF) also exhibit a similar MSNA response during this type of exercise is also an open
10 question. The functional significance of sympathoexcitation during dynamic leg exercise on
11 blood flow in HFrEF, and the relationship between changes in MSNA and $\text{VO}_{2\text{peak}}$ and the
12 effects of exercise training on related mechanisms remain to be established. In healthy men and
13 women the independent effect of aging on the MSNA response during dynamic leg cycling has
14 yet to be addressed definitively.

15
16 **CONCLUSIONS**

17 In summary, sympathetic activity at rest, evaluated directly by microneurography, varies
18 widely between young and older healthy subjects and HFrEF patients. MSNA responses to
19 exercise are a function of exercise type, intensity and posture. In the HFrEF population, baseline
20 fitness level, etiology of HFrEF, and presence of sleep apnea also influence sympathetic nerve
21 firing rates. The inverse relationship between exercise capacity and changes in MSNA during
22 cycling in middle-aged subjects with and without HFrEF supports a potential role for exercise

1 training in reducing sympathoexcitation in those at most risk. The contributions of altered
2 sympathoinhibitory (cardiopulmonary baroreflex) or exaggerated sympathoexcitatory (muscle
3 metaboreflex and mechanoreflex; arterial chemoreflex; cardiopulmonary baroreflex) reflexes
4 during exercise in human HFrEF remain to be elucidated. This represents an important avenue
5 for future research in order to identify more effective targeted exercise interventions and
6 therapies.

Draft

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Table 1. Effects of exercise on muscle sympathetic nerve activity (MSNA) in healthy subjects.

Reference	Population	Exercise Intervention	Nerve Studied	Effects on MSNA during exercise
Mark et al. 1985	14 men; 6 women 20-56 years of age	Static HG (30% MVC)	Fibular	↑ burst frequency and total MSNA during second minute of exercise
Victor et al. 1987	22 men; 3 women 18-30 years of age	Dynamic HG (30% MVC)	Fibular	↔; ↑ only when ischemia added
Saito and Mano 1991	8 men 21±2 years of age	1-leg cycling ($\leq 30\% \text{VO}_{2\text{peak}}$); 1-leg static knee extension (20%MVC)	Tibial	↓ ↑
Saito et al. 1993	8 men 24.5±3.3 years of age	2-leg cycling (20, 40, 60, 75% of $\text{VO}_{2\text{peak}}$)	Median	↓ <40% $\text{VO}_{2\text{peak}}$; ↑ >40% $\text{VO}_{2\text{peak}}$
Ray et al. 1993	16 men 19-22 years of age	1-leg dynamic knee extension (20, 30, 40 Watts)	Fibular	↓ is greatest at mild levels
Batman et al. 1994	30 men 27±1 (19-40 years)	Dynamic HG (25%MVC) Static and dynamic HG (40% MVC)	Fibular	↑ % total activity after 6 min during prolonged dynamic HG (25%) for 30 min ↑ % total activity during 1 and 2 min dynamic HG and only second minute of static HG
Hansen et al. 1994	7 men 21-43 years of age	1-leg static toe extension (20% MVC)	Fibular	↑ burst frequency and total MSNA during second minute of exercise in both R and L nerves
Stickland and Miller 2008	7 men 33±1 (28-35 years)	Dynamic HG (50% MVC)	Fibular	↑ during normoxia; inhibited by hyperoxia
Ichinose et al. 2008	13 men; 2 women 28±1 years of age	2-leg graded cycling (mild to exhaustion 10-190 Watts)	Median	Decrease during mild; ↑ > moderate exercise
Katayama et al. 2011	6 men 22.5±1 years of age	2-leg cycling (40 and 60% $\text{VO}_{2\text{peak}}$)	Median	↔ at 40% $\text{VO}_{2\text{peak}}$; ↑ at 60% $\text{VO}_{2\text{peak}}$; ↑ both with hypoxia

Table 1. Summary of MSNA studies during exercise. Mean $\pm$ Standard error. MSNA, muscle sympathetic nerve activity; MVC, maximum voluntary contraction; VO₂peak, peak oxygen uptake; HG, handgrip; R, right; L, left.

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Table 2. Effects of exercise on muscle sympathetic nerve activity (MSNA) in patients with heart failure with reduced ejection fraction (HFrEF)

Reference	Population	Exercise Intervention	Nerve Studied	Effects on MSNA during exercise
Sterns et al. 1991	9 HFrEF; 53±5 8 control; 55±5	Static HG (30%MVC)	Fibular	↑ in HFrEF and controls ↑ during PHGI in controls and ↓ in HFrEF
Silber et al. 1998	12 HFrEF men; 58±2 10 control men; 64±2	Prolonged Dynamic HG (25%MVC)	Fibular	↑ in HFrEF in first 2 min of HG; ↔ in healthy controls; ↑ total activity at 20 min HG
Notarius et al. 2001	14 HFrEF (1F) (51±4) 10 control (1F) (48±4)	Static HG (30% MVC); Dynamic HG (10, 30, 50% MVC)	Fibular	↑ in HFrEF during all HG and PHGI (30% static and 50% dynamic only) ↔ in healthy controls
Middlekauff et al. 2004	12 HFrEF (49±2) 13 control (47±3)	Dynamic HG (20% MVC); Passive exercise	Fibular	↑ in HFrEF both models; ↓ or ↔ in healthy controls
Murai et al. 2009	16 HFrEF (5F) (62±3) 13 control (4F) (61±3)	Static HG (30% MVC)	Fibular	>↑ in HFrEF both multi and single unit MSNA; ↑ in single unit firing probability in HFrEF
Notarius et al. 2014	11 HFrEF (2F) (61±3) 11 control (2F) (55±2)	1-leg cycling moderate intensity	Fibular	Exercise MSNA inversely correlated with VO ₂ peak independent of resting MSNA
Kuniyoshi et al. 2014	HFrEF Control (50% F)	Static HG (30% MVC)	Fibular	No correlation between MSNA during static HG and VO ₂ peak in HFrEF
Notarius et al. 2015	16 HFrEF (4F) (62±3) 13 healthy (3F) (56±2)	1-leg cycling No resistance; 50% of peak VO ₂	Fibular	↑ in HFrEF ↓ in healthy controls Exercise MSNA inversely correlated with VO ₂ peak

Table 2. Abbreviations as in Table 1. HFrEF, heart failure due to reduced ejection fraction; F, female; PHGI, post-handgrip ischemia

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FIGURE LEGENDS

Figure 1. MSNA varies with age in the control subjects but with peak exercise capacity or VO_2peak in the HFrEF patients. MSNA recordings of two healthy control subjects at rest of differing age: a 60 year old (top left) and a 40 year old (bottom left), both with normal peak VO_2 ; and two HFrEF patients of similar age (40's), one with a virtually normal exercise capacity of 93 percent of predicted (top right) and one with a very low peak VO_2 (bottom right). MSNA, muscle sympathetic nerve activity; VO_2peak , peak oxygen uptake. Each multifibre MSNA nerve burst is marked.

Figure 2. Relationships between resting muscle sympathetic nerve activity (MSNA) burst frequency, MSNA during second minute (2') of submaximal (50% of VO_2peak) leg cycling exercise and percent of predicted peak oxygen uptake (VO_2 peak) presented as a three-dimensional plot of individual data points and regression plane; the slope, or edge of the plane, relating VO_2 peak to exercise-induced MSNA is significant ($P=0.03$); the slope, or edge of the plane, relating VO_2 peak to resting MSNA is not ($P=0.93$). For exercise-induced MSNA adjusted for baseline, $r=0.59$; $P=0.02$. Reprinted from Notarius et al. J.Am.Coll.Cardiol. 2014, with permission.

Figure 3. Divergent MSNA burst frequency and incidence responses to dynamic exercise in HFrEF patients and control subjects.

Top Panel: Mean change in mean heart rate (HR) from baseline during mild and moderate dynamic leg exercise. HR increased significantly in response to increasing exercise intensity during the second minute of one-legged cycling in both healthy control (*white bars*) and heart failure

1 (*black bars*) subjects (+, $P < 0.003$ for 50% vs zero load cycling) with no between-group difference
2 ($P = 0.10$).

3 Bottom Panel: Mean change in MSNA burst frequency (bursts/min) from baseline during mild and
4 moderate dynamic leg exercise. MSNA increased decreased significantly during both exercise
5 intensities in healthy control subjects (*white bars*) but decreased significantly during both exercise
6 intensities in heart failure patients (*black bars*) (*, $P < 0.001$ HFrEF vs control). There was no
7 significant difference in MSNA between exercise intensities within groups ($P = 0.78$). Adapted from
8 Notarius et al. J. Physiol. 2015, with permission.

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Healthy Control **5 sec.****MSNA = 47 bursts/min
VO₂peak = 100% predicted****HFrEF Patient** **MSNA = 41 bursts/min
VO₂peak = 93% predicted** **MSNA = 31 bursts/min
VO₂peak = 90% predicted** **MSNA = 70 bursts/min
VO₂peak = 33% predicted**

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